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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/884,877	06/20/2001	Henricus Petrus Joseph Te Riele	065691-0230	3654

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EXAMINER	
WOITACH, JOSEPH T	

ART UNIT	PAPER NUMBER
1632	

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14

Please find below and/or attached an Office communication concerning this application or proceeding.

File

Office Action Summary

Application No.	Applicant(s)
09/884,877	Riele et al.
Examiner	**Art Unit**
Joseph Woitach	1632

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136 (a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on Oct 6, 2002 .

2a) ☐ This action is **FINAL.** 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 24-49 is/are pending in the application.

4a) Of the above, claim(s) 34-49 is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 24-33 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claims _____ are subject to restriction and/or election requirement.

Application Papers

9) ☒ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on Jun 20, 2001 is/are a) ☒ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11) ☐ The proposed drawing correction filed on _____ is: a) ☐ approved b) ☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12) ☒ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. §§ 119 and 120

13) ☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a) ☐ All b) ☐ Some* c) ☐ None of:

1. ☐ Certified copies of the priority documents have been received.
2. ☐ Certified copies of the priority documents have been received in Application No. _____ .
3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

*See the attached detailed Office action for a list of the certified copies not received.

14) ☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

a) ☐ The translation of the foreign language provisional application has been received.

15) ☒ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO-1449) Paper No(s). 2
4) ☐ Interview Summary (PTO-413) Paper No(s). _____
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other:

DETAILED ACTION

This application filed June 20, 2001 is a continuation in part of 09/147,712, filed February 23, 1999, now abandoned, which is a national stage filing of PCT/EP95/02980, filed July 26, 1995.

Applicants' preliminary amendment filed June 20, 2001 and the amendment filed April 15, paper number 11, have been received and entered. The specification has been amended. Claims 24-49 are pending and currently under examination.

Election/Restriction

Applicant's election with traverse of Group I, claims 24-33, in Paper No. 13 is acknowledged. The traversal is on the ground(s) that the Examiner has not adequately established that search and examination of the entire application constitutes an undue burden. This is not found persuasive because for a proper restriction **one** of two standards must be met: 1) the inventions must be independent or distinct, and 2) there must be a serious burden on the examiner if restriction is not required of a proper restriction has been met (MPEP 806.04 and 808.02). In this case, Applicant does not contest that the inventions are not distinct, only that there is there would be an undue burden to search and examine the entire application, and for this reason the restriction is found proper. With respect to arguments of burden of search and examination, MPEP 808.02 states that 'the Examiner, in order to establish reasons for insisting

upon restriction, must show by appropriate explanation of one of the following: (A) Separate classification, (B) Separate status in the art when classifiable together, or (C) a different field of search'. In this case, each of the separate restriction groups has a different classification, and specific differences between the embodiments encompassed by each of the groups were set forth in the restriction requirement (see restriction requirement, pages 2-3, paper number 12). Applicants' arguments are not found persuasive because the separate classifications and the different subject matter encompassed by the restriction groups require separate searches of the art for the unique embodiments of each of the groups and different considerations of the relevant art as they apply to the unique embodiments.

The requirement is still deemed proper and is therefore made FINAL.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Claims 24-49 are pending. Claims 34-49 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected inventions, there being no allowable generic or linking claim. Applicant timely traversed the restriction (election) requirement in Paper No. 13. Claims 24-33 are currently under examination.

Oath/Declaration

The oath or declaration is defective. A new oath or declaration in compliance with 37 CFR 1.67(a) identifying this application by application number and filing date is required. See MPEP §§ 602.01 and 602.02.

The oath or declaration is defective because:

Non-initialed and/or non-dated alterations have been made to the oath or declaration. See 37 CFR 1.52(c). On page one the title of the invention and the post office information of Niels DeWind has been modified.

Additionally, the application was filed with an unexecuted declaration and a preliminary amendment to the specification. However, while the declaration indicates the amendments to the PCT application, the executed declaration does not refer to the preliminary of the instant application. The substitute declaration should indicate the preliminary amendment submitted in the filing of the instant application.

Specification

The disclosure is objected to because of the following informalities: the preliminary amendment filed June 20, 2001, amends the specification to indicate related applications, however the specification as filed contains the same information in the first line of the specification. One of the two recitations of the priority information should be deleted.

Appropriate correction is required.

Information Disclosure Statement

The information disclosure statement filed June 20, 200, filed with paper number 2, indicates that the cited references were not provided because they were provided in parent application 09/147,712. The references from 09/147,712 have been obtained and reviewed. A signed copy of the IDS has been include with the instant action.

Additionally, the listing of references in the specification is not a proper information disclosure statement. See specification, pages 29-44. 37 CFR 1.98(b) requires a list of all patents, publications, or other information submitted for consideration by the Office, and MPEP § 609 A(1) states, "the list may not be incorporated into the specification but must be submitted in a separate paper." Therefore, unless the references have been cited by the examiner on form PTO-892, they have not been considered.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claim 33 is rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The invention is drawn to a mammalian cell wherein both Msh2 alleles are inactivated. More specifically, the specification teaches that the term dMsh2-9 cell refers to a mouse ES cell wherein both Msh2 alleles are disrupted (page 17, middle of paragraph 45). The dMsh2-9 cell was derived from a mouse embryonic stem cell in which one of the Msh2 alleles was disrupted using homologous recombination and was generated by prolonged selective culture under conditions (page 16, paragraph 45). The specification teaches that the cell termed dMsh2-9 contained the disrupted Msh2 allele which was present in the parent cell (generated by homologous recombination) and lost the wild type copy of the Msh2 gene (page 17, middle of paragraph 45). The specification teaches that the method used to derive the dMsh2-9 cell in which both Msh2 alleles are disrupted also resulted in a cells in which only one allele was disrupted, termed sMsh2-55 and sMsh2-42. The specification teaches that the method used to derive the two different cells, dMsh2-9 and sMsh2-55, results in cells which have different properties as evidenced by their sensitivity to MNNG in culture (page 23, paragraph 66) and the affect on phenotype (tumor type and frequency) when used to generate a knockout mouse model (page 28, Table 2). Though the mouse embryonic stem cell generated by homologous recombination to alter the first Msh2 allele could be made following the teachings provided by the instant specification, the methods used to generate a disruption in both alleles results in various cell types. For example dMsh2-9, sMsh2-42 and sMsh2-55 each display different phenotypic characteristics though they were generated by the same method. Further, while the specification indicates that the wild type copy of Msh2 has been lost in the dMsh2-9 cell, the

specification fails to describe what portion of the allele was determined as lost other specific changes that have occurred which provide the unique characteristics to the dMsh2-9 cell described in the instant specification. In summary, dMsh2-9 was generated by a process during which unknown modifications to the original mouse embryonic stem cell were made. Further, the method used to generate dMsh2-9 does not uniquely generate a homogeneous type of cell and the method clearly generates cell types which are different from dMsh2-9. Since the methods used result in various cell types, and the structure of dMsh2-9 is not described such that distinguishing and defining features are completely described, dMsh2-9 represents a unique cell which can not be reproduced. Claim 33 is drawn specifically to dMsh2-9, therefore the dMsh2-9 cell are essential to the claimed invention. dMsh2-9 must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. Since the method used to produce dMsh2-9 result in uncharacterized alterations of the cells in culture the dMsh2-9 cell line is not so obtainable or available. However, the requirements of 35 U.S.C. 112, regarding "how to make", may be satisfied by a deposit of the dMsh2-9 cell line. There is no indication in the specification that the dMsh2-9 cell or cell line was deposited. However, if a deposit was made and if the deposits was made under the terms of the Budapest Treaty, then an affidavit or declaration by Applicant, or a statement by an attorney of record over his or her signature and registration number, stating that the specific cell lines have been deposited under the Budapest Treaty and that the cell lines will be irrevocably and without restriction released to the public upon the issuance of a patent, would satisfy the deposit requirement.

It the deposit has not been made under the Budapest Treaty, then in order to certify that the deposit meets the criteria set forth in 37 CFR 1.801-1.809, Applicant may provide assurance of compliance by an affidavit or declaration, or by a statement by an attorney of record over his or her signature and registration number, showing that

(a) during the pendency of this application, access to the invention will afforded to the Commissioner upon request;

(b) all restrictions upon availability to the public will be irrevocably removed upon granting of the patent;

(c) the deposit will be maintained in a public depository for a period of 30 years or 5 years after the last request of for the effective life of the patent, whichever is longer; and,

(d) a test of viability of the biological material at the time of deposit (see 37 CFR 1.807); and,

(e) the deposit will be replaced if it should ever become inviable.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

(b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 24, 26, 27 are rejected under 35 U.S.C. 102(b) as being anticipated by Reenan *et al.* (Genetics 132:975-985).

Reenan *et al.* teach that yeast have a homolog of mammalian msh2 (page 975, second column). Further, Reenan *et al.* describe the cloning and characterization of the yeast msh2 gene. To characterize the msh2 gene, Reenan *et al.* teach a method of disrupting the msh2 gene in yeast (page 976, methods section) (claims 24 and 27). Further, Reenan *et al.* teach that both alleles of the msh2 gene can be modified in the yeast to form homozygous diploids (page 980, bottom of first column)(claim 26). Reenan *et al.* teach that disruption of msh2 results lower spore viability in yeast having single disruptions (page 981, first column) and increased gene conversion when homozygous diploids are analyzed (page 981, second column). In the characterization of the affects of disrupting msh2 in yeast, Reenan *et al.* conclude that msh2 is part of the nuclear mismatch repair system (page 983, first full paragraph).

Claims 29, 30 and 32 are rejected under 35 U.S.C. 102(b) as being anticipated by Umar *et al.* (JBC 269:14367-14370).

Umar *et al.* teach several human colorectal and endometrial cancer cell lines which demonstrate defects in the mismatch system of the cell. In the analysis of the various cell lines, Umar *et al.* teach the status of the msh2 gene in each of the cell lines. Umar *et al.* teach that the

endometrial cancer cell line HEC59 had one allele of the msh2 gene which was mutated (page 14369, top of second column)(claim 29 and 30) and that the colorectal cancer line LoVo had both alleles disrupted (claims 29 and 32). Further, Umar *et al.* teach that each of these cell lines, LoVo and HEC59, display a replication error phenotype which is associated with mismatch repair system of the cell (summary in Table I).

It is noted that instant claims are drawn to cell made by the method set forth in claim 29, and thus, a product by process, however where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. Whether the rejection is based on "inherency" under 35 USC 102 or "prima facie obviousness" under 35 USC 103, jointly or alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. *In re Best, Bolton, and Shaw*, 195 USPQ 430, 433 (CCPA 1977) citing *In re Brown*, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972). In the instant case, the method of claim 29 (and claims 25, 27 and 28) encompasses inactivating the msh2 gene in a mammalian cell. The cells disclosed in Umar *et al.* are mammalian cells in which the msh2 gene is disrupted. Further, Umar *et al.* teach that the disruption of the msh2 gene results in a mismatch phenotype in these cells. Therefore, while the cells taught by Umar *et al.* are not made by the method set forth in claim 29, the cells would

anticipate the instant claims because they meet the structural limitations of cells that would result from practicing the method set forth in claim 29.

Claims 29, 30 and 32 are rejected under 35 U.S.C. 102(a) as being anticipated by Orth *et al.* (PNAS 91:9495-9499).

Orth *et al.* teach several human ovarian cancer cell lines which demonstrate defects in the mismatch system of the cell. In the analysis of the various cell lines, Orth *et al.* teach the status of the msh2 gene in the ovarian cell line 2774 had one mutated allele of the msh2 gene and that the second wild type allele was lost during tumorigenesis (summarized in abstract and page 9498, first full paragraph)(claim 29 and 32). Further, Orth *et al.* teach that normal non-tumor cells from the patient which the 2774 cell line was derived contained only one mutant msh2 allele and one wild type allele (summarized in abstract and page 9498, and third and full paragraph)(claim 29 and 30). Finally, Orth *et al.* teach that alterations of the msh2 gene are associated with genomic instability (page 9496, second column and figure 2).

It is noted that instant claims are drawn to cell made by the method set forth in claim 29, and thus, a product by process, however where, as here, the claimed and prior art products are identical or substantially identical, or are produced by identical or substantially identical processes, the PTO can require an applicant to prove that the prior art products do not necessarily or inherently possess the characteristics of his claimed product. Whether the rejection is based on "inherency" under 35 USC 102 or "prima facie obviousness" under 35 USC 103, jointly or

alternatively, the burden of proof is the same, and its fairness is evidenced by the PTO's inability to manufacture products or to obtain and compare prior art products. *In re Best, Bolton, and Shaw*, 195 USPQ 430, 433 (CCPA 1977) citing *In re Brown*, 59 CCPA 1036, 459 F.2d 531, 173 USPQ 685 (1972). In the instant case, the method of claim 29 (and claims 25, 27 and 28) encompasses inactivating the msh2 gene in a mammalian cell. The cells disclosed in Umar *et al.* are mammalian cells in which the msh2 gene is disrupted. Further, Umar *et al.* teach that the disruption of the msh2 gene results in a mismatch phenotype in these cells. Therefore, while the cells taught by Umar *et al.* are not made by the method set forth in claim 29, the cells would anticipate the instant claims because they meet the structural limitations of cells that would result from practicing the method set forth in claim 29.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later

invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 24-30 and 32 are rejected under 35 U.S.C. 103(a) as being unpatentable over Varlet *et al.*, Genbank accession number X81143, and Berns *et al.* (US Patent 5,789,215 or WO 93/04169).

At the time of the claimed invention Varlet *et al.* teaches that homologs of Msh2 were known for several species including the mammals mouse and human (summary in figure 1). Further, Varlet *et al.* summarize the prior art and teach that Msh2 in lower eukaryote was associated with mismatch repair. In higher eukaryote, Varlet *et al.* summarizes that mutations and absence of the Msh2 gene in humans is associated with hereditary non-polyposis colorectal cancer (HPNCC), and the predisposition of a patient to tumor formation (pages 5723-4, bridging paragraph). The predisposition to tumor formation associated with the loss of Msh2 in tumors was consistent with the loss mismatch repair in *in vitro* systems where the biochemistry of Msh2 was previously analyzed and described in the prior art (page 5723, first paragraph). To analyze homologs of Msh2 previously described in the art, Varlet *et al.* describe the isolation of the mouse Msh2 coding sequence (bridging pages 5724-5725) and indicate that the mouse sequence was deposited as X81143 (see Genbank listing). In characterizing the endogenous expression of Msh2 in the mouse Varlet *et al.* demonstrate a ubiquitous mRNA expression pattern throughout most of the tissues tested (figure 3). Varlet *et al.* note that the expression pattern in the mouse is consistent with replication, however in view of the limited spectrum of tumors in patients with

hereditary non-polyposis colorectal cancer the tissue specific role of Msh2 'in HNPCC patients is surprising' (page 5727, top of first column). Varlet *et al.* propose several possible explanations for the observed results and indicate 'further study of the biochemistry (in Xenopus egg lysates) and of the genetics (in mouse) of mismatch repair will shed new light on its [Msh2] role in the maintenance of the integrity of eukaryotic genome, and in the development of cancer (page 5727, final paragraph). Varlet *et al.* indicate the types of further research required to address their hypothesis explaining the limited spectrum of tumor formation *in vivo* in patients with mutant Msh2 alleles, however they do not provide the specific guidance to provide a model system. At the time of filing transgenic mice were used to provide *in vivo* models of human diseases. Both references by Berns *et al.* teach a method of generating transgenic animals wherein a gene of interest is disrupted. Specifically, Berns *et al.* teach that a gene of interest in the genome of a mouse can be disrupted by generating a targeting construct wherein a homologous sequence to the gene of interest is modified by the insertion of a selectable marker such as hygromycin ('215 column 5, lines 26 and exemplified in figures and working examples), and using said targeting construct to achieve disruption of the gene of interest in a mouse embryonic stem cell through homologous recombination methodology in the mouse embryonic stem cell ('215 summarized in abstract). Berns *et al.* teach that modifications to the genome can be an insertion, deletion, or substitution within the gene of interest wherein the site specific integration of the targeting construct results in a modification which disrupts expression of the gene product ('215 column 4, lines 59-67). Finally, Berns *et al.* teach that upon generating a mouse embryonic stem cell with

the desired alteration in its genome, the embryonic stem cell can be used to generate knock-out transgenic animals wherein one or both copies of the genes of interest contain the modification ('215 paragraph bridging columns 15 and 16).

Varlet *et al.* teach that further *in vivo* analysis of the role of Msh2 is required and that the study of the genetics in mice will provide further insight on the development of cancer, therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to take the specific Msh2 sequences disclosed and suggestion by Varlet *et al.* to make mouse embryonic stem cells with a disrupted Msh2 gene using the knock-out methodology described by Berns *et al.* One having ordinary skill in the art would have been motivated to use the methods described by Berns *et al.* to disrupt the expression of the Msh2 gene because Varlet *et al.* teach that it is the absence of expression of the Msh2 gene that was associated with the mutant phenotype in cells and in tumors isolated from patients. Further, by providing a mouse embryonic stem cell with the endogenous Msh2 gene expression disrupted using the methods of Berns *et al.*, a knock-out animal can be produced to test the hypothesis set forth by Varlet *et al.* There would have been a reasonable expectation of success to generate a mouse embryonic stem cell with a disrupted Msh2 gene using the methods of Berns *et al.* given the successful results demonstrated by Berns *et al.* for the Rb gene in the working examples and his teaching that other knock-out animals have been made using similar methodology.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

Claims 29, 30 and 31 are rejected under 35 U.S.C. 103(a) as being unpatentable over Varlet *et al.*, Genbank accession number X81143, and Berns *et al.* (US Patent 5,789,215 or WO 93/04169) in view of Promega Protocols and Applications Guide.

As discussed above, claims 29 and 30 are obvious over the teachings of Varlet *et al.*, Genbank accession number X81143, and Berns *et al.* Briefly, Berns *et al.* provide specific methods for disrupting expression of a gene of interest in embryonic stem cells. Varlet *et al.* teach that Msh2 gene is a DNA mismatch repair gene and disruption of it's expression results in loss of DNA repair activity *in vitro* and is associated with the progression of carcinogenesis in vivo. The mouse Msh2 sequence isolated and characterized by Varlet *et al.* is disclosed in Genbank accession number X81143. Varlet *et al.* specifically teaches that analysis of Msh2 in mice will provide further insight into understanding of the role Msh2 *in vivo* and as a model of cancer development in humans.

Berns *et al.* provide a detailed description for modifying a gene of interest in the genome of animal by the use of 'targeting DNA' (column 4, lines 15-26). Specifically, Berns *et al.* teach that DNA modification of a gene can be of several types including insertions (column 4, lines 59-64), and in particular the 'insertion of the selectable marker into the coding region of the target gene' (column 5, lines 4-5). Further, Berns *et al.* teaches a variety of selectable marker genes which can be used in making targeting constructs including hygromycin (column 5, lines 19-26, in particular line 26). Berns *et al.* repeat if a selectable marker is used to disrupt a target gene expression, 'then the selectable marker can be cloned into targeting DNA corresponding to

coding sequence in the target DNA' (column 5, lines 44-43). Finally, Berns *et al.* teach that the methods used to construct the targeting DNA are standard laboratory procedures with respect to recombinant DNA technology such is found in Maniatis *et al.* or Sambrook *et al.* (column 11, lines 43-54, and column, lines 37-41). However, neither Varlet *et al.* nor Berns *et al.* specifically teach the insertion of a marker between codons 588 and 589 of Msh2. A restriction map of Msh2 indicates multiple restriction sites in the coding sequence of Msh2, including a SnaB1 site starting at base pair 1801 which is at codon 588 (see Genbank accession number X81143). Promega teaches that the SnaB1 enzyme and recognition site were known at the time of filing. Further, Promega teach that other endonuclease such as Eco105I were known. Eco105I is an isoschizomer of SnaB1, and can be used to provide the appropriate DNA overhang ends for the insertion of a second marker sequence into the SnaB1 site. The use and insertion of an isoschizomer sequence destroys the original recognition site creating a unique polynucleotide sequence for restriction analysis for the confirmation of said insertion. The SnaB1 site provides a convenient insertion site for a selectable marker in the coding region of Msh2 therefore, it would have been *prima facie* obvious to one having ordinary skill in the art at the time the invention was made to take the specific Msh2 sequences disclosed and suggestion by Varlet *et al.* to make mouse embryonic stem cells with a disrupted Msh2 gene using the knock-out methodology described by Berns *et al.* More specifically, it would have been obvious to use the SnaB1 site because it is in the coding region of Msh2 and provides a specific insertion site for a marker gene such as hygromycin into Msh2. One having ordinary skill in the art would have

been motivated to use the SnaB1 site because this site is in the coding region of Msh2. Further, the use of the SnaB1 site allows for the use of isoschizomers and the generation of unique restriction sites for the generation and analysis of the targeting DNA and the analysis of the resulting genome once the target DNA is inserted into the genome (described in the methods of Berns *et al.* column 15, lines 20-30). There would have been a reasonable expectation of success to generate a mouse embryonic stem cell with a disrupted Msh2 gene at codons 588-589 because the SnaB1 enzyme was known and the methods of generating recombinant plasmids and screening genomic DNA by Southern bot analysis was conventional at the time of filing.

Thus, the claimed invention as a whole was clearly *prima facie* obvious.

Conclusion

No claim is allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Joseph Woitach whose telephone number is (703)305-3732.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Deborah Reynolds, can be reached at (703)305-4051.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group analyst Dianiece Jacobs whose telephone number is (703) 308-2141.

Papers related to this application may be submitted by facsimile transmission. Papers should be faxed via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center numbers are (703)308-4242 and (703)305-3014.

Joseph T. Woitach

Jo Woitach
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